Amendments to the Specification:

Please amend the first paragraph on page 1, after the title, as follows:

This application is a continuation of ~~co-pending~~ application Serial No. 09/564,142, filed May 3, 2000 (now abandoned); which is a continuation application of application Serial No. 08/859,555, filed May 20, 1997 (now abandoned); which was a continuation application of application Serial No. 08/411,062; filed March 27, 1995 (now U.S. Patent No. 5,631,358); which was a continuation application of Serial No. 07/214,297; filed July 1, 1988 (now abandoned).

Please amend Paragraphs [0019]-[0020] on page 11, lines 14-25, and page 12, lines 1-10, as follows:

[0019] Examples of tissue-specific early nodulin regulatory regions are found in the 5' flanking region of the soybean (Glycine max) Enod2a and Enod2b genes which encode N-75. The Enod2a regulatory region extends about 1 kb 5' from the transcription start of the genes. The regulatory region contains the nucleotide sequence from ~~Table 1~~ Figure 2 extending from about nucleotide 520 to about nucleotide 1565. The Enod2b regulatory region extends about 1 kb 5' from the transcription start of the gene, from about nucleotide 1320 to about nucleotide 2365, as in ~~Table 2~~ Figure 3. These regulatory regions direct the expression of a downstream gene in a tissue-specific manner in the developing root nodule.

[0020] An additional example of a tissue-specific early nodulin gene regulatory region is the DNA sequence common to the 5' flanking regions of the soybean Enod2a and Enod2b genes. This regulatory element contains DNA sequence as given in ~~Table 1~~ Figure 2, extending from about nucleotide 1050 to about nucleotide 1565, or given in ~~Table 2~~ Figure 3, extending from about nucleotide 1850 to about nucleotide 2365. This regulatory region directs the expression of a downstream structural gene in a tissue-specific manner in the developing root nodule.

Please amend Paragraph [0024] at page 14, lines 6-16, as follows:

BRIEF DESCRIPTION OF THE FIGURES

[0024] FIG. 1 gives a schematic restriction endonuclease map of the soybean Enod2a and Enod2b genes, and the regions which flank them. Schematic diagrams of CHA-6 (containing the Enod2a gene) and CHA-9 (containing the Enod2b gene) are given. The regions sequenced ~~(Tables 1 and 2)~~ of both clones are ~~indicated~~ provided in Figures 2A-2D (Enod2a; DNA is SEQ ID NO:1; encoded polypeptides are SEQ ID NO:2 and SEQ ID NO:3) and Figures 3A-3D (Enod2b; DNA is SEQ ID NO:4; encoded polypeptides are SEQ ID NO:5 and SEQ ID NO:6). The region of approximately 100% homology between the two genomic clones ~~is indicated~~, as ~~are~~ well as the regions of the clones homologous to the Enod2 cDNA clone, can be determined by alignment. Restriction endonucleases are labelled as follows: H=HindIII, B=BamHI, S=Sau3A, E=EcoRI.

Please add the following paragraphs following Paragraph [0024] at page 14, line 17:

[0024a] FIG. 2A-D provides the nucleotide sequence of the Enod2a genomic clone (SEQ ID NO:1) and the amino acid sequences for SEQ ID NOs:2-3.

[0024b] FIG. 3A-D provides the nucleotide sequence of the Enod2b genomic clone (SEQ ID NO:4) and the amino acid sequences for SEQ ID NOs:5-6.

[0024c] BRIEF DESCRIPTION OF THE SEQUENCES

[0024d] SEQ ID NO:1 is the nucleotide sequence of the Enod2a genomic clone in FIG. 2A-D.

[0024e] SEQ ID NO:2 is the amino acid sequence encoded by nucleotides 1654-2494 of SEQ ID NO:1.

[0024f] SEQ ID NO:3 is the amino acid sequence encoded by nucleotides 1751-2678 of SEQ ID NO:1.

[0024g] SEQ ID NO:4 is the nucleotide sequence of the Enod2b genomic clone in FIG. 3A-D.

[0024h] SEQ ID NO:5 is the amino acid sequence encoded by nucleotides 2456-3264 of SEQ ID NO:4.

[0024i] SEQ ID NO:6 is the amino acid sequence encoded by nucleotides 2553-3380 of SEQ ID NO:4.

Please amend Paragraphs [0026]-[0027] on page 14, line 22, through page 17, line 7, as follows:

[0026] The Enod2 gene described herein is an early nodulin gene of soybean (Glycine max), which encodes nodulin polypeptides with an apparent molecular weight of about 75 kDa, nodulin 75 (N-75). Two such genes are exemplified by the Enod2a and Enod2b genes which are identified by the DNA sequences given in ~~Tables 1 and 2~~ Figures 2A-D and 3A-D, respectively.

[0027] The Enod2 regulatory region is the DNA sequence 5' and adjacent to the Enod2 coding sequence, which includes promoter sequences and promoter-associated sequences and controls tissue-specific expression of the Enod2 genes in soybean. The regulatory region extends about 1 kb upstream from the transcription start site of an Enod2 gene. All the signals required from tissue-specific regulated gene expression are contained in the approximately 1 kb 5' flanking region. Within this stretch of DNA are sequences with homology to the TATA and CAAT consensus sequences of eukaryotic promoters, and the nodulin gene consensus sequences a and c (V. P. Mauro et al. (1985), supra), which are believed to be involved in the regulation of the expression of nod genes expressed later than Enod2 during nodulation. There are also sequence motifs with homology to the SV40 enhancer core consensus sequence which are found in the regulatory region of the soybean Enod2a gene. There may also be other sequence elements which modulate the level of gene expression, which respond to stimuli from the B. japonicum, or which determine the tissue-specific expression in the developing soybean root nodule after inoculation with Bradyrhizobium japonicum. The expression of Enod2 genes controlled by the Enod2 regulatory region is tissue-specific in that it is limited to the cortex of developing soybean root nodules. The Enod2 regulatory region controls early gene expression in the developing root nodule of soybean with expression beginning at about 7 days after seed planting and inoculation. Expression is induced by contact with soybean nodulating bacteria, such as B. japonicum. Enod2 gene expression also occurs in the ineffective nodules induced by strains of Rhizobium fredii. The Enod2a regulatory region is a DNA sequence which includes promoter sequences and promoter-associated sequences and controls the expression of the soybean Enod2a gene. The Enod2a regulatory region extends about 1 k:b upstream from the Enod2a gene transcription start. This region is specifically identified by the DNA sequence in ~~Table 1~~ Figure 2A-D from about

nucleotide 520 to about nucleotide 1565. The Enod2b regulatory region is a DNA sequence which includes promoter sequences and promoter-associated sequences and controls the expression of the soybean Enod2b gene. The Enod2b regulatory region extends about 1 kb upstream from the Enod2b gene transcription start. This region is specifically identified by the DNA sequence in ~~Table 2~~ Figure 3A-D from about nucleotide 1320 to about nucleotide 2365. These regulatory regions direct tissue-specific expression of a downstream structural gene, such that the gene is selectively expressed in the inner cortex of the developing root nodule in soybean. The Enod2 common regulatory region is the DNA sequence extending about 500 bases upstream of the transcription start site of an Enod2 gene. The Enod2 common regulatory region is exemplified by the homologous sequences of Enod2a and Enod2b extending from about nucleotide 1050 to about nucleotide 1565 (~~Table 1~~ Figure 2A-D), and about nucleotide 1850 to about nucleotide 2365 (~~Table 2~~ Figure 3A-D), respectively. This common regulatory region controls tissue-specific expression of downstream genes in the cortex of developing soybean root nodules.

Please amend Paragraph [0035] at page 22, line 5, through page 23, line 6, as follows:

pEnod2 was isolated from a cDNA library prepared with 21-day-old soybean root nodule RNA, using RNA from 10-day-old nodules as a probe. Thus, pEnod2 represents an early nodulin cDNA clone. The early nodulin encoded by pEnod2 was identified by hybrid-selecting nodule mRNA and translating in vitro. Two polypeptides, with apparent Mrs of 75000, were found and were each called N-75. The mRNAs homologous to pEnod2 were only about 1200 nucleotides long, with the capacity to encode a protein of at most about 45 kDa. Therefore the soybean-specific insert of pEnod2 was sequenced and the amino acid sequence of N-75 was deduced. Two ORFs of similar size were found (labelled ORF1 and ORF2 on ~~Tables 1 and 2~~ Figures 2A-D and 3A-D), one with about 20 methionines and the other a proline-rich sequence, with a repeating heptameric sequence. Because of the anomalous migration on SDS-polyacrylamide gels and because of the labelling patterns the two N-75s, it was concluded that the proline-rich coding sequence (ORF1) was that of N-75. It is believed that N-75 is involved in nodule morphogenesis because of its proline content and because of the pattern of expression in the

developing nodule. N-75 appears at about day 7 after sowing and inoculation, and increases through day 13; mRNA continues to be present at least through day 21. N-75 is also produced in the developing ineffective nodule of soybean inoculated with Rhizobium fredii USDA257. That leads to the conclusion that typical nodule structure with successful infection of the root by rhizobia is not absolutely required for Enod2 expression.

Please amend Paragraph [0037] at page 23, line 14, through page 24, line 3, as follows:

Two soybean genomic clones corresponding to pEnod2 have been isolated and the DNA sequences of the coding and flanking regions have been determined (~~Tables 1 and 2~~ Figures 2A-D and 3A-D). The genes, termed Enod2a and Enod2b, are essentially homologous from about 600 bp 5' to the ATG translation start codon through the coding region, which is not interrupted by introns, and through some 500 bp of 3' flanking sequence. Comparison of the genomic clones with the Enod2 cDNA sequence indicates that one or both of these genes are expressed in the developing root nodule. S1 mapping of the transcription start site led to the conclusion that the Enod2a start site is at nucleotide 1543 .+-.20 as shown in ~~Table 1~~ Figure 2A-D, and the Enod2b start site is deduced to be similarly located at about nucleotide 2350, as shown in ~~Table 2~~ Figure 3A-D.

Please amend Paragraphs [0054]-[0056] at page 36, line 4, through page 37, line 18, as follows:

[0054] Subsequently, portions of p4.5BE and p10.2 were subcloned into pUC18 and pUC19 vectors and sequenced as described in Example 2. The DNA sequences of the portions of p4.5BE and p10.2 containing the Enod2 genes are displayed in ~~Tables 1 and 2~~ Figures 2A-D and 3A-D. The coding regions and the deduced amino acid sequences of both genes are shown.

EXAMPLE 4

Sequence Analysis of the Enod2a and Enod2b Genes of Soybean

[0055] Standard techniques, as described above, were used for the sequencing of the Enod2a and Enod2b genomic sequences. The coding region of each of these genes is an uninterrupted sequence of 930 bp. ~~Table 1~~ Figure 2A-D gives the DNA sequence of the coding region of the

Enod2a gene along with about 1650 bp of 5' flanking sequence and about 360 bp of 3' flanking sequence. The coding region and about 600 of 5' flanking sequence of the Enod2b gene is almost identical in sequence to that of the Enod2a gene as shown in ~~Table 2~~ Figure 3A-D; a total of about 2450 of 5' flanking sequence and about 470 bp of 3' flanking sequence of the Enod2b gene are also presented in ~~Table 2~~ Figure 3A-D. It was noted that the two genes were 100% homologous over the coding regions, and almost 100% homologous in the approximately 600 bp of 5' flanking DNA extending to a Sau3A site at positions 1048 in Enod2a and 1852 in Enod2b, and in the 3' flanking DNA that has been sequenced.

[0056] Analysis of the sequence of the cDNA clone pEnod2 and the sequences revealed that there were two open reading frames (ORF1 and ORF2) of similar length; both are noted in ~~Tables 1 and 2~~ Figures 2A-D and 3A-D. The anomalous migration in SDS-polyacrylamide gel electrophoresis experiments led, in part, to the conclusion that the ORF1 is the actual coding sequence of the Enod2 genes encoding N-75. The polypeptide encoded by ORF1 is rich in proline, and proline-rich polypeptides are known to exhibit aberrant behavior during SDS-polyacrylamide gel electrophoresis (J. W. Freytag et al. (1979), supra). The second line of reasoning was that one of the hybrid-selected translation products was devoid of methionine; ORF1 has only one methionine codon (at the translation start) while the alternate ORF1 contained about 20 methionine codons, and therefore its translation product should have been labelled readily with ^{35}S-methionine.

Please replace Figures 2A and 3A with replacement Figures 2A and 3A, which are attached following the Remarks section along with annotated Figures 2A and 3A showing changes made.